



Substrate-Induced Conformational Dynamics of the Dopamine Transporter

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and conformation probability of these states. These results allow us to design novel ligands with longer RTs, improving their potential for clinical use.

1712-Pos

Purification of an Engineered Membrane Protein FhuA for Size-Dependent Separation

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FhuA is a porin protein found within the outer membrane of *Escherichia coli* bacteria. Similar to other porin proteins, FhuA forms a beta barrel. At the resting state, the channel formed by the barrel is blocked by a plug formed by its N-terminal domain, as well as several extracellular loops. The deletion of the amino acid sequences corresponding to the plug as well as four extracellular loops results in the FhuA $\Delta C/\Delta 4L$ mutant, which has a less occluded lumen. The goal of the project is to use the FhuA mutant as a channel for size-selective separation once embedded into artificial membranes. As a first step toward the goal, we optimized the purification process of the FhuA $\Delta C/\Delta 4L$ mutant so that it can be obtained with high yield and purity. It was found that the optimal conditions involved using freshly transformed *E. coli* BL21 (DE3) Rosetta Omp 8 from an agar plate, skipping solubilization of the inner membrane, including 5 mM imidazole during binding to the nickel resin, and use of a 30 kDa concentrator. The purified protein was then used to fabricate FhuA nanosheets supported on PES membranes, with filtration tests showing a molecular weight cutoff of 1-3 kDa. Permeability tests have shown that the FhuA demonstrates a flux significantly higher than that of commercial nanofiltration membranes with similar size pores, indicating that the FhuA mutant has the potential to be used for bioseparations applications.

1713-Pos

Effects of Membrane Heterogeneity and Aggregation on the Lateral Migration and Colocalization of Proteins

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Biological membranes are complex mixtures of lipids and proteins and are thought to be laterally heterogeneous. While there are still contentions about stabilities and size scales of these heterogeneous domains, their utility is thought to be essential in protein sorting. Spectroscopic evidence is now emerging on protein partitioning in membranes that closely resemble these biological conditions, providing the opportunity for careful quantitative validation of simulation results. Employing coarse-grained (MARTINI) molecular dynamics simulations and Flory-Huggins type theoretical models of multicomponent lipid membranes with single and multiple copies of transmembrane proteins, we first validate the observed protein colocalization and then go on to investigate the effects of membrane heterogeneity, protein sequence and protein aggregation on the observed migration and colocalization of proteins. We report that the coarse-grained simulations show significant limitations in imparting the experimentally observed partitioning of transmembrane domain of the Linker for Activation of T cells (trLAT) protein and its mutants, and the simulated protein partitioning shows disparities in monomeric and multimeric systems due to protein aggregation.

1714-Pos

Substrate-Induced Conformational Dynamics of the Dopamine Transporter

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The dopamine transporter is a member of the neurotransmitter:sodium symporters (NSSs), which are responsible for termination of neurotransmission through Na⁺-driven reuptake of neurotransmitter from the extracellular space. The coordinated conformational rearrangements related to the transport mechanism have so far been poorly understood. Here we have probed the global Na⁺- and dopamine-induced conformational dynamics of the wild-type *Drosophila melanogaster* dopamine transporter using hydrogen-deuterium exchange mass spectrometry. We identify Na⁺- and dopamine-induced changes in specific regions of the transporter, suggesting their involvement in protein conformational transitions. Furthermore, we detect novel ligand-dependent slow cooperative fluctuations of helical stretches in several domains of the

transporter, which could be a novel molecular mechanism that assists in the transporter function. Our results provide a framework for understanding the molecular mechanism underlying the function of NSSs by revealing the first detailed insight into the state-dependent conformational changes associated with the alternating access model of a eukaryotic NSS.

1715-Pos

The Presence of a Lipopolysaccharide Substrate Stimulates Lateral Gating in LptD

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Lipopolysaccharides (LPS) provide the outer membrane of Gram-negative bacteria with a strong protective barrier. The Lpt machinery is responsible for the transport of LPS molecules across the periplasm, culminating in insertion by the outer-membrane proteins LptD/E. Structures for the LptD/E complex reveal an unprecedented 26-strand beta-barrel with an N-terminal jelly roll domain and LptE as the plug domain for LptD. The primary model for LPS insertion posits that a transmembrane beta-jelly roll bridge guides LPS across the periplasm. Upon reaching the outer membrane, LptD ushers LPS through a lateral gate between the first and last beta strands of its beta-barrel domain. While this model is generally accepted, the molecular details of LPS insertion by LptD remain elusive. In order to gain clarity of these details we have performed over 10 microseconds of equilibrium MD simulation of LptD/E systems and performed free energy calculations of LptD lateral gate opening with and without substrate bound. Equilibrium simulations reveal that substrate binding in the N-terminal domain induces lateral gate opening, and furthermore, free energy calculations reveal that substrate binding reduces the energetic barrier for lateral gate strand separation.

1716-Pos

How Shape, Flexibility, and Crowding affect Curvature Sensing and Generation by Generic Scaffolding Proteins

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Bin/Amphiphysin/Rvs (BAR) proteins are a family of proteins responsible for initiating and driving membrane deformation orders of magnitude larger than a single protein. Currently, BAR proteins are believed to form a dense protein scaffold that drives membrane remodeling important to many cellular processes. Our simulations of BAR proteins improve our understanding by probing generic features common to the BAR family and adding detail to the protein self-assembly prior to large-scale membrane deformation. We develop a mesoscopic phenomenological model explicitly representing each protein/BAR domain and the membrane. Our generic representation can probe wide span of intrinsic curvature of the BAR family from negatively curved I-BAR to positively curved N- and F- BAR. Beyond that, we investigate fluctuations and flexibility in the intrinsic curvature as protein rigidity has become an increasingly key factor to curvature sensing and generation. Importantly, we use the generic representation to probe the effect of the full-length protein vs. an isolated BAR domain, which has gained increasing interest due to the experimental determined differences in membrane remodeling. In addition, our membrane model can be deformed into variety of membrane shapes (e.g. neck of a tubule), which effects the membrane-bound protein aggregation. We perform a comprehensive study of the effects of protein intrinsic curvature and flexibility, presence of full-length protein and crowding, and membrane shape on generic BAR domain self-assembly and curvature sensing.

1717-Pos

Molecular Determinants of Neisserial Opa Protein Interactions with Human CEACAMs

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Neisseria gonorrhoeae is a unique pathogen in that it not only colonizes human tissue by interactions with host receptors, but these interactions can trigger engulfment of the bacteria even by typically non-phagocytic cells. *N. gonorrhoeae* possess a family of opacity-associated (Opa) proteins, which are 8-stranded β -barrel membrane proteins that bind to human host receptors. The majority of Opa proteins engage select human CEACAMs (carcino-embryonic antigen-like cellular adhesion molecules) specifically, and this interaction can trigger phagocytosis of the bacterium. To date, there are 345 distinct *opa* alleles sequenced; the most prominent differences are in the second and third extracellular loops (hypervariable regions, HV1 and HV2). In addition to contributing to the bacteria's ability to evade the human immune system, these HV regions primarily determine receptor specificity. While the Opa protein family has conserved structural elements, the molecular determinants of receptor